# PATENT COOPERATION TREATY

# PCT

## INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY

(Chapter II of the Patent Cooperation Treaty)

(PCT Article 36 and Rule 70)

| Applicant's or agent's file reference | FOR FURTHER ACTION | See Form PCT/IPEA/416 |
|---|---|---|
| AX02A03/P-WO | | |

| International application No. | International filing date *(day/month/year)* | Priority date *(day/month/year)* |
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| PCT/EP2004/007529 | 08.07.2004 | 08.07.2003 |

International Patent Classification (IPC) or national classification and IPC
C12N5/06, C12N15/79, C12N15/82, C12Q1/68

Applicant
AXIOGENESIS AG et al.

1. This report is the international preliminary examination report, established by this International Preliminary Examining Authority under Article 35 and transmitted to the applicant according to Article 36.
2. This REPORT consists of a total of 13 sheets, including this cover sheet.
3. This report is also accompanied by ANNEXES, comprising:
   a. ☒ *sent to the applicant and to the International Bureau)* a total of 6 sheets, as follows:
      ☒ sheets of the description, claims and/or drawings which have been amended and are the basis of this report and/or sheets containing rectifications authorized by this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions).
      ☐ sheets which supersede earlier sheets, but which this Authority considers contain an amendment that goes beyond the disclosure in the international application as filed, as indicated in item 4 of Box No. I and the Supplemental Box.
   b. ☐ *(sent to the International Bureau only)* a total of (indicate type and number of electronic carrier(s)) , containing a sequence listing and/or tables related thereto, in computer readable form only, as indicated in the Supplemental Box Relating to Sequence Listing (see Section 802 of the Administrative Instructions).

4. This report contains indications relating to the following items:

| | | |
|---|---|---|
| ☒ | Box No. I | Basis of the opinion |
| ☐ | Box No. II | Priority |
| ☒ | Box No. III | Non-establishment of opinion with regard to novelty, inventive step and industrial applicability |
| ☐ | Box No. IV | Lack of unity of invention |
| ☒ | Box No. V | Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement |
| ☐ | Box No. VI | Certain documents cited |
| ☐ | Box No. VII | Certain defects in the international application |
| ☐ | Box No. VIII | Certain observations on the international application |

| Date of submission of the demand | Date of completion of this report |
|---|---|
| 15.06.2005 | 26.08.2005 |

| Name and mailing address of the international preliminary examining authority: | Authorized Officer |
|---|---|
| European Patent Office<br>D-80298 Munich<br>Tel. +49 89 2399 - 0 Tx: 523656 epmu d<br>Fax: +49 89 2399 - 4465 | Hermann, P<br>Telephone No. +49 89 2399-7109 |

# INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY

International application No.
PCT/EP2004/007529

## Box No. I Basis of the report

1. With regard to the **language**, this report is based on the international application in the language in which it was filed, unless otherwise indicated under this item.

   ☐ This report is based on translations from the original language into the following language , which is the language of a translation furnished for the purposes of:
   - ☐ international search (under Rules 12.3 and 23.1(b))
   - ☐ publication of the international application (under Rule 12.4)
   - ☐ international preliminary examination (under Rules 55.2 and/or 55.3)

2. With regard to the **elements*** of the international application, this report is based on *(replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report)*:

**Description, Pages**

| | |
|---|---|
| 1-45 | as originally filed |

**Sequence listings part of the description, Pages**

| | |
|---|---|
| 1-21 | as originally filed |

**Claims, Numbers**

| | |
|---|---|
| 1-52 | received on 15.06.2005 with letter of 15.06.2005 |

**Drawings, Sheets**

| | |
|---|---|
| 1/1 | as originally filed |

☒ a sequence listing and/or any related table(s) - see Supplemental Box Relating to Sequence Listing

3. ☒ The amendments have resulted in the cancellation of:
   - ☐ the description, pages
   - ☒ the claims, Nos. 53, 54
   - ☐ the drawings, sheets/figs
   - ☐ the sequence listing *(specify)*:
   - ☐ any table(s) related to sequence listing *(specify)*:

4. ☐ This report has been established as if (some of) the amendments annexed to this report and listed below had not been made, since they have been considered to go beyond the disclosure as filed, as indicated in the Supplemental Box (Rule 70.2(c)).
   - ☐ the description, pages
   - ☐ the claims, Nos.
   - ☐ the drawings, sheets/figs
   - ☐ the sequence listing *(specify)*:
   - ☐ any table(s) related to sequence listing *(specify)*:

* *If item 4 applies, some or all of these sheets may be marked "superseded."*

**INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY**

International application No.
PCT/EP2004/007529

**Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability**

1. The questions whether the claimed invention appears to be novel, to involve an inventive step (to be non-obvious), or to be industrially applicable have not been examined in respect of:

☐ the entire international application,

☒ claims Nos. 44 (for I.A.)

because:

☒ the said international application, or the said claims Nos. 44 (for I.A.) relate to the following subject matter which does not require an international preliminary examination (specify):

**see separate sheet**

☐ the description, claims or drawings *(indicate particular elements below)* or said claims Nos. are so unclear that no meaningful opinion could be formed *(specify)*:

☐ the claims, or said claims Nos. are so inadequately supported by the description that no meaningful opinion could be formed.

☒ no international search report has been established for the said claims Nos. 37,38,45,46 (all in part)

☐ the nucleotide and/or amino acid sequence listing does not comply with the standard provided for in Annex C of the Administrative Instructions in that:

| | | |
|---|---|---|
| the written form | ☐ | has not been furnished |
| | ☐ | does not comply with the standard |
| the computer readable form | ☐ | has not been furnished |
| | ☐ | does not comply with the standard |

☐ the tables related to the nucleotide and/or amino acid sequence listing, if in computer readable form only, do not comply with the technical requirements provided for in Annex C-*bis* of the Administrative Instructions.

☐ See separate sheet for further details

**Box No. V Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement**

1. Statement

| | | | |
|---|---|---|---|
| Novelty (N) | Yes: | Claims | 1-44, 48-52 |
| | No: | Claims | 45-47 |
| Inventive step (IS) | Yes: | Claims | 1-44, 48-52 |
| | No: | Claims | 45-47 |
| Industrial applicability (IA) | Yes: | Claims | 1-43, 45-52 |
| | No: | Claims | - |

2. Citations and explanations (Rule 70.7):

**see separate sheet**

## Supplemental Box relating to Sequence Listing

**Continuation of Box I, item 2:**

1. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application and necessary to the claimed invention, this report has been established on the basis of:

   a. type of material:

   ☒ a sequence listing

   ☐ table(s) related to the sequence listing

   b. format of material:

   ☒ in written format

   ☒ in computer readable form

   c. time of filing/furnishing:

   ☒ contained in the international application as filed

   ☒ filed together with the international application in computer readable form

   ☐ furnished subsequently to this Authority for the purposes of search and/or examination

   ☐ received by this Authority as an amendment on

2. ☐ In addition, in the case that more than one version or copy of a sequence listing and/or table(s) relating thereto has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

3. Additional observations, if necessary:

Re Item I

**Basis of the report**

The amendments filed with the response to the Written Opinion dated June 15th, 2005, within the prescribed time limit, do not introduce subject-matter which extends beyond the content of the application as filed, and therefore meet the requirements of Article 34(2)(b) PCT.

Re Item III

**Non-establishment of opinion with regard to novelty, inventive step and industrial applicability**

Claims 37, 38, and 43-47 lack clarity (Article 6 PCT) because the products to which the claims relate are not defined by characteristic features but by a method which should lead to their identification leaving therefore the reader in doubt as regards the intended scope of protection. Moreover the description is silent as to a potential common or essential characteristic feature for the claimed product. With respect to claims 45 and 46, the lack of clarity is such that the claims could not be searched over their entire scopes and the search and therefore the opinion as regards novelty, inventive step and industrial applicability (Article 33 PCT) for the subject-matters of claims 45 and 46 have been limited to the compounds and pharmaceutical compositions clearly defined in the description as promoting or modulating cell growth and/or differentiation, i.e. retinoic acid and pharmaceutical compositions comprising the same (see the examples).

Moreover claims 37 and 38 do not provide any steps for the manufacture of the drug the claims relate to, leading therefore to a lack of clarity (Article 6 PCT). The steps given in the wording of the claims correspond to screening steps and therefore the opinion as regards novelty, inventive step and industrial applicability (Article 33 PCT) for the subject-matters of claims 37 and 38 is given in so far as their respective subject-matter relate to screening methods.

Claim 44 relates to subject-matter considered by this Authority to be covered by the provisions of Rule 67.1(iii) PCT. Consequently, no opinion will be formulated with respect to the industrial applicability of the subject-matter of said claim (Article 34(4)(a)(i) PCT).

Re Item V

**Reasoned statement with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement**

The following documents D are referred to in this communication; the numbering will be adhered to in the rest of the procedure:

**D1**: US 2003/008836 A1 (Goldspink Geoffrey) 9 January 2003

**D2**: Müller M. *et al.* - "Selection of ventricular-like cardiomyocytes from ES cells *in vitro*" - 2000 - *FasebJ.*, **14**: 2540-2548

**D3**: Wobus A. M. *et al.* - "Retinoic acid induces expression of the ventricular 2.1 kb myosin-light-chain2 promoter during *in-vitro* cardiogenesis of embryonic stem cells" - 1995 - *Circulation*, **92**(8): I-114

**D4**: Wobus A. M. *et al.* - "Retinoic acid accelerates embryonic stem cell-derived cardiac differentiation and enhances development of ventricular cardiomyocytes" - 1997 - *J. Mol. Cell. Cardiol.*, **29**: 1525-1539

**D5**: Klug M. G. *et al.* - "Genetically selected cardiomyocytes from differentiating embryonic stem cells form stable intracardiac grafts" - 1996 - *J. Clin. Invest.*, **98**: 216-224

**D6**: Bronstein I. *et al.* - "Chemiluminescent reporter gene assays: sensitive detection of the GUS and SEAP gene products" - 1994 - *Biotechniques*, **17**: 174-177

**D7**: US 2002/042096 A1 (Rosen Craig A. *et al.*) 11 April 2002

**2. Novelty**

2.1 The present application does not meet the requirements of Article 33(1) PCT, because the subject-matters of claims 45-47 are not new in the sense of Article 33(1) and (2) PCT.

2.1.1 Claims 45 and 46 relates to products define by a process. Such claims are new

only if the product per se is new however, retinoic acid is a well known inducer of cell differentiation (cf. **D4** p. 1527 paragraph bridging left to right-hand columns - p. 1529 left-hand column 1st §, and Fig. 1) which can be identified by the claimed methods, therefore, compositions comprising retinoic acid anticipates the novelty of claims 45 and 46 (Article 33(2) PCT). Furthermore, the use of said compositions comprising the retinoic acid anticipates the novelty of claim 47 (Article 33(2) PCT). Therefore D4 anticipates the novelty of claims 45-47.

2.2 No document at hand discloses:

- the method of claim 1 -12;
- the reporter gene construct of claim 13;
- the cell, cell aggregate, tissue, organ, implant and non-human animal of claim 14, 15, 16, 17, 18 and 19 respectively;
- the composition of matter of claim 20;
- the array of independent claim 21;
- the uses of claim 22, 48 and 52;
- the method of claims 23-41;
- the kit of independent claim 42;
- the method of independent claims 43 and 44; and
- the vector of claims 49-51.

The subject-matter of claims 1-44 and 48-52 is therefore novel, said claims thus meet the requirements of Article 33(2) PCT.

**3. Inventive step**

3.1 The subject-matters of claims 1-44 and 48-52 are considered to meet the requirements of Article 33(3) PCT, the reasons being as follows:

3.1.1 Document **D4** is considered to be the closest relevant prior art for claim 1 and discloses a method for the monitoring of cell differentiation. Said method includes

the following steps (cf. **D4** p. 1527 paragraph bridging left to right-hand columns - p. 1529 left-hand column 1st §, and Fig. 1):

- culturing embryonic stem (ES) cells capable of differentiating and forming embryoid bodies, which contain at least one recombinant nucleic acid molecule comprising a reporter gene encoding a specific and detectable product upon cell differentiation (i.e. lacZ gene under the control of MLC-2v promoter region), under conditions allowing differentiation of the cells (i.e. retinoic acid); and
- determining the amount or activity of the reporter gene product.

The method of claim 9 differs from the method of document **D4** by the fact that the reporter gene encodes a product that is secreted. The effect provided by said difference is that it is easier to detect/monitor cell differentiation without killing the differentiated cells. Furthermore the use of said reporter assay allow the quantification of differentiated cells.

In view of **D4**, the problem to be solved by the method of claim 9 can be considered as the provision of an improved method for the detection and quantification of cells undergoing differentiation.

The solution to said problem, as contained in claim 1 is the use of a different reporter gene which product is released in the culture medium such as secreted alkaline phosphatase (SEAP) or alpha-amylase as suggested in dependent claim 9.

Document **D6** discloses all the advantages of using a SEAP as a reporter gene: i) the cell population remains intact since the detection is performed on the culture supernatant; ii) the detection of SEAP is quantitative and iii) highly sensitive especially when detected with a chemiluminescent assay (cf. **D6** p. 172 right-hand column last § - p. 173 left-hand column 1st §, p. 174 paragraph linking the left-and the right hand columns, Fig. 2., results and discussion).

Moreover, document **D7** further supports the use of SEAP reporter system and describes a reporter assay wherein the reporter gene (secreted alkaline phosphatase) is put under the control of different promoters and enhancers, the choice of said promoter / enhancer depending on the event to be monitored (cell activation, proliferation and/or differentiation)(cf. **D7** examples 32-36).

However neither D6 nor D7 indicate the potential use of SEAP reporter system to quantify differentiated cells. D7 use SEAP reporter system for measuring event in cell lines which are almost at the final stage of their differentiation and without distinction between the proliferation and the differentiation event, and document D6 reports the use of a SEAP reporter system under CMV promoter which therefore cannot be used to quantitate differentiation events. Therefore the prior art at hand would not have motivated the skilled person to deviate from the teaching of D4 and arrive to the solution provided in independent claim 1. Furthermore the example presented in the description confirm that the method of independent claim 1 solves the problem posed. Therefore the method of claim 1 meet the requirements of Article 33(3) PCT.

3.1.2 The subject-matter of dependent claims 2-12 which further define specific embodiments of the novel and inventive method of claim 1 is also novel and inventive. Thus dependent claims 2-12 are hence also considered to meet the requirements of Article 33(1) , (2) and (3)PCT.

3.1.3 The reporter gene construct disclosed in examples 32-35 of document D7 render D7 closest prior-art for the subject-matter of present independent claim 13. The reporter gene construct of claim 13 differs from that of document D7 by the fact that the promoter used in said construct lead to the expression of the soluble reporter molecule only when cells are differentiating whereas in document D7 the promoter used reflects the activation of the Jaks-STATs pathway. Moreover none of the documents at hand during examination, including D7, indicates to the skilled person that the promoter used in D7 could be replaced by promoter inducible under differentiation. Therefore and for identical reasons as those given under point 3.1.1

above, the reporter gene construct of claim 13, is considered to be based on an inventive concept, and the products comprising the said reporter gene construct are thus also inventive as well as the vector of claims 49-51 and the use of the reporter gene construct or of the promoter as contained in claims 48 and 52 respectively.

Therefore claims 13-21, 42 and 48-52 meet the requirements of Article 33(3) PCT.

3.1.4 A similar reasoning as that of point 3.1.1 above applies mutatis mutandis to the methods of claims 23-36, 39-41, 43 and 44. Said claims 23-36 and 39-41, 43 and 44 thus meet the requirements of Article 33(3) PCT.

3.1.5 Claims 37 and 38, in so far as they have been examined (cf. ITEM III above) i.e. as being related to screening methods, are also considered inventive and meet therefore the requirements of Article 33(3) PCT.

**4. Clarity**

4.1 Independent claims 13-21, 42, 45, 46 are unclear (Article 6 PCT) because the products to which said claims relate are defined by reference to method claims and not by characteristic features of the products themselves, therefore leaving the reader in doubt as to the real scope of the claims (see also PCT International Search and Examination Guidelines 5.31-5.33). In particular, claim 13 refers back to claims 1-12; however some of these claims do not directy relate to any feature of the reporter gene construct.

4.2 Although claims independent claims 1 and 23; 37, 38 and 39; and 43 and 44 have been drafted as separate independent claims, they appear to relate effectively to the same subject-matter and to differ from each other only with regard to the definition of the subject-matter for which protection is sought and in respect of the terminology used for the features of that subject-matter. The aforementioned claims therefore lack concise-

ness. Moreover, lack of clarity of the claims as a whole arises, since the plurality of independent claims makes it difficult, if not impossible, to determine the matter for which protection is sought, and places an undue burden on others seeking to establish the extent of the protection (Article 6 PCT).

4.3 Claims 37-39 are unclear (Article 6 PCT) because they define independent methods which however are identical to the method of independent claim 23 (identical steps) and only differ by respective results to be achieved which in turn, depend completely on the properties of the compound which is to be identified by the said method (see also PCT International search and Examination Guidelines 5.35).

## 5. Industrial applicability (Article 33(4) PCT)

5.1 For the assessment of the present claim 44 on the question whether it is industrially applicable, no unified criteria exist in the PCT Contracting States. The patentability can also be dependent upon the formulation of the claims. The EPO, for example, does not recognize as industrially applicable the subject-matter of claims to method of doing business.

## 6. Further comments

6.1 Dependent claim 4 relates to the use of "embryonic stem cells or multipotent adult progenitor cells", therefore encompassing the use of cells originating from human embryos. By consequence the scope of claims 1-54 encompasses the use of human embryos for industrial and/or commercial purposes.

Applicant's attention is drawn to the fact that, upon entry into the regional phase, patentability of claims relating to human embryos may underlie restrictions based on moral grounds. The EPO, for example, does not recognize as patentable the subject-matter of claims to the cloning of human beings, the modification of the germ line identity

of human beings and the use of human embryos for industrial or commercial purposes (Article 53(a) and Rule 23d EPC).

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Axiogenesis AG
Our Ref.: AX02A03/P-WO



## Claims

1. A method of monitoring cell differentiation comprising:
   (a) culturing cells capable of differentiating into at least one particular cell type containing at least one recombinant nucleic acid molecule comprising a reporter gene encoding a product that is secreted upon cell differentiation, or maintaining a non-human animal comprising such cells, under conditions allowing differentiation of the cells; and
   (b) quantifying the differentiated cells by determining the amount or activity of the reporter gene product either within a body fluid of said transgenic non-human animal or the cell culture medium.

2. The method of claim 1, wherein said recombinant nucleic acid molecule comprises at least one cell type-specific regulatory sequence operably linked to said reporter gene.

3. The method of claim 1 or 2, wherein said cells are or are derived from stem cells.

4. The method of claim 3, wherein said stem cells are embryonic stem cells or multipotent adult progenitor cells (MAPCs).

5. The method of any one of claims 1 to 4, wherein said reporter gene product comprises a secretory leader sequence.

6. The method of any one of claims 2 to 5, wherein said regulatory sequence comprises a promoter and/or enhancer element.

7. The method of any one of claims 1 to 6, wherein said cell type is selected from the group consisting of connecting fibroblasts, stromal cells, endothelial cells, glial cells, neural cells, neuronal cells, hematopoietic cells, smooth muscle cells, skeletal muscle cells, epithelial cells, and cardiac cells.

8. The method of claim 6 or 7, wherein said promoter or enhancer is selected from the group consisting of aMHC, MLC2V, VE-cadherin, Tie-2, Flk-1, Flt-1, GFAP, alpha-

1-tubulin and collagen 2 promoter or enhancer.

9. The method of any of claims 1 to 8, wherein said reporter gene product is secreted alkaline phosphatase (SEAP) or alpha-amylase.

10. The method of any one of claims 1 to 9, wherein said recombinant nucleic acid molecule further comprises a selectable marker expressed by multi- or pluripotent cells.

11. The method of any one of claims 1 to 10, wherein said cells form cell aggregates or tissue-like aggregates derived from different cell types.

12. The method of any one of claims 1 to 11, wherein said cells form embryoid bodies (EBs).

13. A reporter gene construct for monitoring cell differentiation by quantifying the differentiated cells comprising a recombinant nucleic acid molecule as defined in any one of claims 1 to 12.

14. A cell as defined in any one of claims 1 to 12 comprising a reporter gene construct of claim 13, wherein said cell is capable of differentiating into at least one particular cell type.

15. A cell aggregate of at least one cell type obtainable by the method of any one of claims 1 to 12.

16. A tissue obtainable by the method of any one of claims 1 to 12 or comprising cells of claim 14 or a cell aggregate of claim 15.

17. An organ comprising a tissue of claim 16, a cell of claim 14 or a cell aggregate of claim 15.

18. An implant or transplant comprising an organ of claim 17, a tissue of claim 16, a cell of claim 14 or a cell aggregrate of claim 15.

19. A non-human animal comprising a reporter gene construct of claim 13, a cell of claim 14, a cell aggregate of claim 15, a tissue of claim 16 or an organ of claim 17.

20. A composition of matter comprising a reporter gene construct of claim 13, a tissue of claim 16, cells of claim 14 or a cell aggregate of claim 15.

21. An array comprising a solid support and attached thereto or suspended thereon cells of claim 14, a cell aggregate of claim 15 or a tissue of claim 16.

22. Use of an apparatus for analyzing the array of claim 21.

23. A method of obtaining and/or profiling a modulator of cell differentiation comprising:

(a) contacting a test sample comprising a cell of claim 14, a cell aggregate of claim 15, a tissue of claim 16 or an organ of claim 17 or a non-human animal of claim 19 with a test substance; and

(b) determining the effect of the test substance on the amount of the reporter gene product or activity compared to a control sample or animal.

24. The method of claim 23, wherein said contacting step further includes contacting said test sample or animal with at least one second test substance in the presence of said first test substance.

25. The method of any one of claims 1 to 12 or 23 to 24, wherein a compound known to activate or inhibit the differentiation process is added to the culture medium or animal.

26. The method of any one of claims 23 to 25, wherein the test substance is a therapeutic agent.

27. The method of any one of claims 23 to 26, wherein the test substance is a mixture of therapeutic agents.

28. The method of any one of claims 23 to 27, wherein preferably in a first screen said test substance is comprised in and subjected as a collection of test substances.

29. The method of claim 28, wherein said collection of test substances has a diversity of about 103 to about 105.

30. The method of claim 29, wherein the diversity of said collection of test substances is successively reduced.

31. The method of any one of claims 23 to 30, which is performed on an array.

32. The method of any one of claims 1 to 12 or 23 to 31, wherein said one or more cells are genetically engineered to (over)express or inhibit the expression of a target gene.

33. The method of any one of claims 1 to 12 or 23 to 32, wherein said one or more cells or tissue are contained in a container.

34. The method of any one of claims 1 to 12 or 23 to 33, comprising taking 3 or more measurements, optionally at different positions within the container.

35. The method of claim 33 or 34, wherein said container is a well in a microtiter plate.

36. The method of claim 35, wherein said microtiter plate is a 24 , 96 , 384 or 1586 well plate.

37. A method of obtaining and manufacturing a drug which promotes or inhibits formation of specific cell types comprising the steps of any one of claims 23 to 36, wherein an enhanced or reduced level or activity of the reporter gene product is indicative for the drug.

38. A method of manufacturing an agent which supports wound healing and/or healing of damaged tissue comprising the steps of the method of any one of claims 23 to 37, wherein an enhanced level or activity of the reporter gene product is indicative for said agent.

39. A method of determining toxicity, preferably teratogenicity, embryotoxicity, chronic or acute toxicity of a compound comprising the steps of the method of any one of claims 23 to 37, wherein a reduced or enhanced level or activity of said reporter gene product is indicative for the toxicity of the compound.

40. The method of any one of claims 23 to 39, further comprising modifying said substance to alter, eliminate and/or derivatize a portion thereof suspected causing toxicity, increasing bioavailability, solubility and/or half-life.

41. The method of any one of claims 23 to 40, further comprising mixing the substance isolated or modified with a pharmaceutically acceptable carrier.

42. A kit useful for conducting a method of any one of claims 1 to 12 or 23 to 41, containing a reporter gene construct of claim 13 or a cell of claim 14, and optionally standard compounds, like cell culture media, selection agents, detection agents for the reporter molecule and control samples.

43. A method of conducting a drug discovery comprising:

(a) providing one or more assay systems of any one of claims 1 to 12 or 23 to 41 for identifying a modulator of cell differentiation; and/or

(b) conducting therapeutic profiling of modulators identified in step (a), or further analogs thereof, for efficacy and toxicity in animals of claim 19; and

(c) formulating a pharmaceutical preparation including one or more modulators identified in step (b) as having an acceptable therapeutic profile.

44. A method of conducting a target discovery business comprising:

(a) providing one or more assay systems of any one of claims 1 to 12 or 23 to 41 for identifying modulators of cell differentiation;

(b) (optionally) conducting therapeutic profiling of modulators identified in step (a) for efficacy and toxicity in animals of claim 19; and

(c) licensing, to a third party, the rights for further drug development and/or sales for modulators identified in step (a), or analogs thereof.

45. A modulator of cell differentiation such as growth and tissue formation promoting identified according to the method of any one of claims 23 to 41.

46. A pharmaceutical composition for use in the modulation of cell differentiation comprising a modulator identified according to the method of any one of claims 23 to 41.

47. A method of making a pharmaceutical composition for use in modulating cell differentiation comprising mixing a modulator of cell differentiation identified according to a method of any one of claims 23 to 41 with a suitable diluent or carrier.

48. Use of a reporter gene construct of claim 13, a cell of claim 14, a cell aggregate of claim 15, a tissue of claim 16, an organ of claim 17, the implant or transplant of claim 18, a non-human animal of claim 19, the composition of claim 20, an array of claim 21 or the apparatus of claim 22 in drug discovery or pharmacokinetic or pharmacological profiling.

49. A vector comprising the promoter region of the mouse alpha myosin heavy chain gene or of the ventricular myosin regulatory light chain gene, and operably linked thereto a heterologous DNA sequence, which encodes secreted alkaline phosphatase protein (SEAP).

50. The vector of claim 49, wherein said promoter comprises the nucleotide sequence of SEQ ID NO: 1 or 2, or a fragment thereof.

51. The vector of claim 49 or 50 comprising the nucleotide sequence of SEQ ID NO: 3.

52. Use of a promoter region of the mouse alpha myosin heavy chain gene or of the ventricular myosin regulatory light chain gene for the specific expression of heterologous DNA sequences during embryogenesis or cell development in a method of any one of claims 1 to 12 or 23 to 41.